

APPENDIX

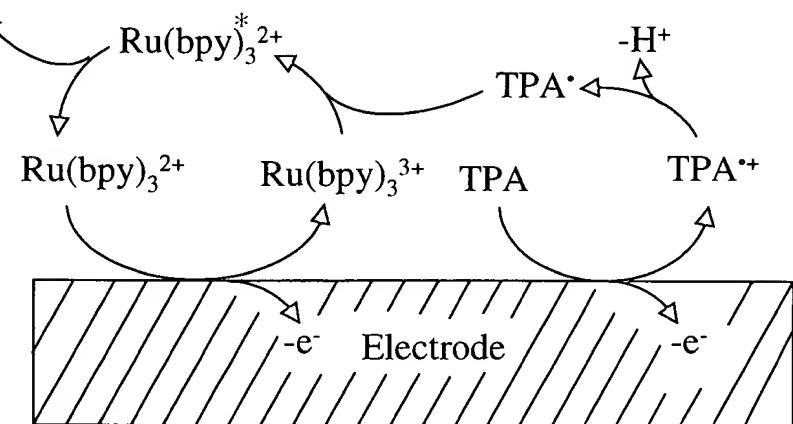
Figure Description

- 1 Overview of Solid-Phase Binding Assays
- 2 Proposed Mechanism of ECL Detection with $\text{Ru}(\text{bpy})_3^{2+}$
- 3 Current State of the Art in ECL Assays (Magnetic Bead-Based Assays)
- 4 Schematic Diagram of Present Invention (Multi-Array Multi-Specified Testing)
- 5 Comparison of ECL Assays Using Beads with Assays Using Reagents Immobilized on Electrodes (One Embodiment of the Present Invention)
- 6 Schematic Illustrations of (6a) the Leventis Invention, (6b) the Shibue Invention, and (6c) the Combination of Shibue and Leventis



Mechanism of ECL Detection With $\text{Ru}(\text{bpy})_3^{2+}$

Photon (620 nm)



TPA = Tripropylamine

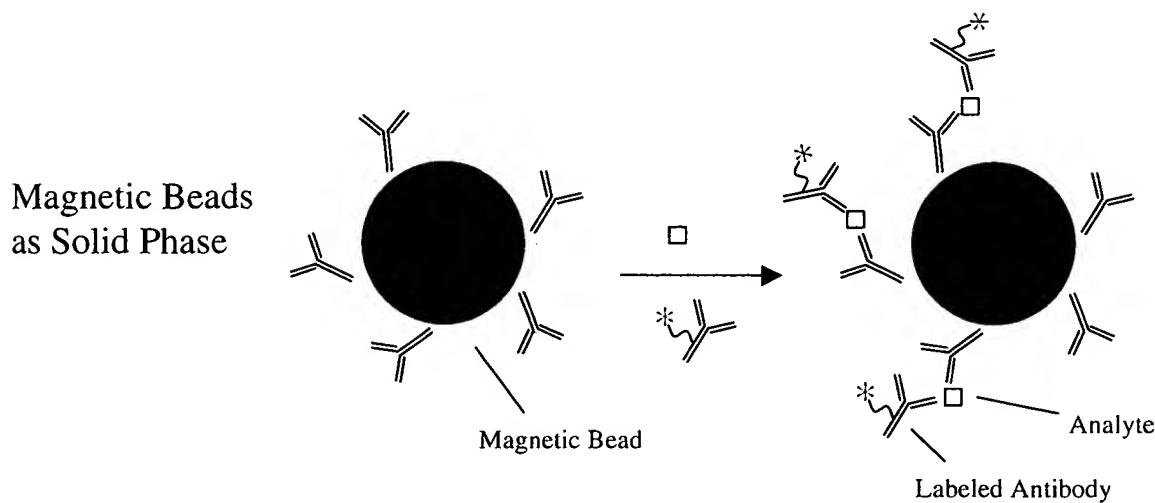
- Reaction is triggered by an applied electrical potential.
- Reaction is confined to the surface of the electrode.
- Electrode must be capable of oxidizing/reducing ECL moiety and coreactant.

FIG. 2

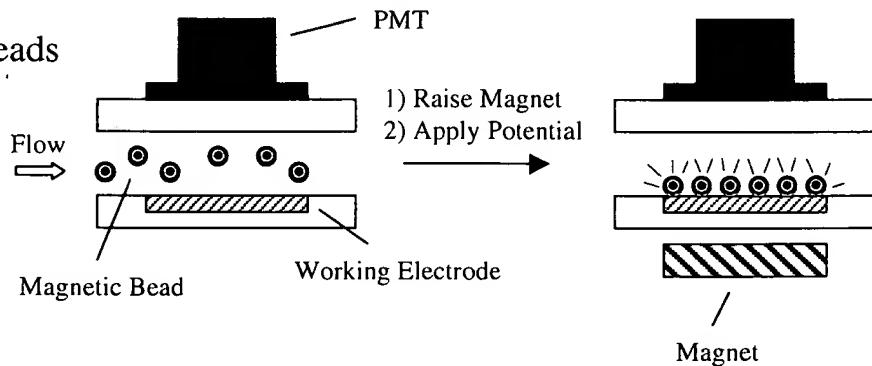
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Current State of the Art in ECL Assays

ORIGEN™ Technology



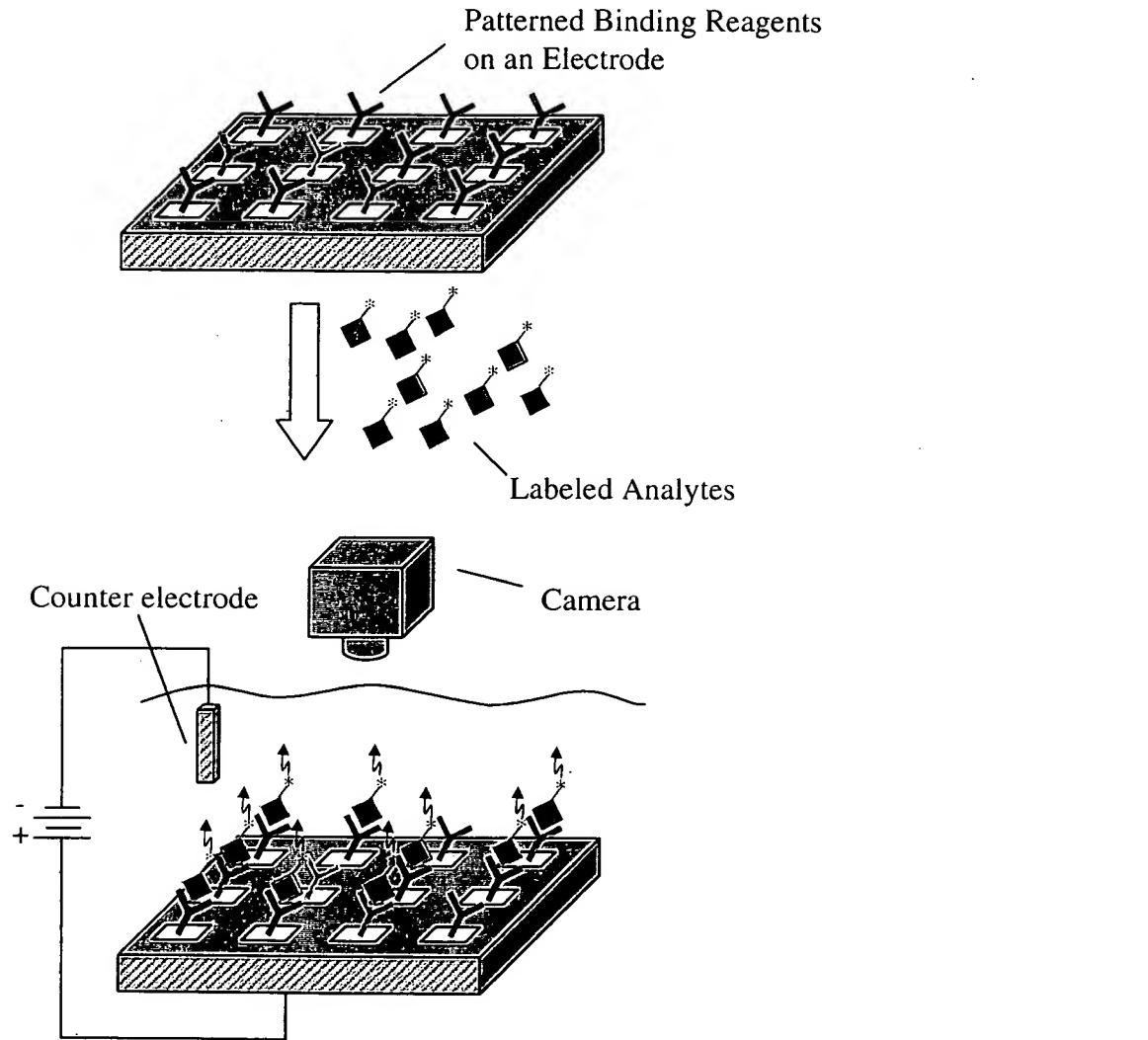
Use of a Magnet to Collect Magnetic Beads at an Electrode



- Magnetic Beads as Solid Phase for Binding Assays.
- Capture of Magnetic Beads on Electrode Surface.
- Scrupulously Clean Electrode.
- Multiple Use Electrode
- One Assay at a Time.

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Multi-Array Multi-Specific ECL Testing

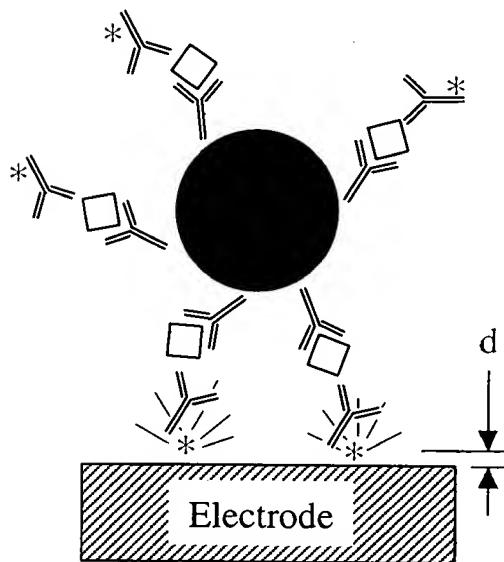


- Binding reagents are immobilized directly on the working electrode.
- Binding reagents for different analytes are patterned on the electrode.
- A plurality of different analytes in a sample are determined simultaneously by imaging ECL generated at the surface.
- The method does not require the use of more than one type of ECL label.
- Alternate embodiments:
 - 1) Multiple electrodes supporting different binding reagents: different analytes determined by sequential triggering of electrodes.
 - 2) Binding reagents patterned on a surface other than the electrode: second surface is placed in contact with electrode to trigger ECL.

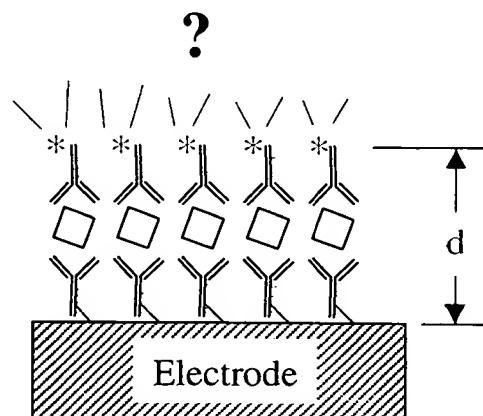
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Comparison of ECL Assays Using Magnetic Beads with Assays Using Binding Reagents Immobilized Directly on an Electrode

Excitation of ECL During Magnetic Bead-Based Assay



Excitation of ECL During Assay Using Binding Reagents Immobilized on a Electrode

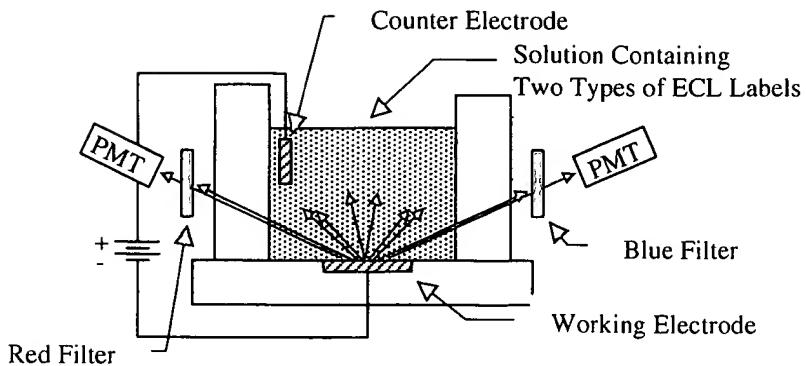


- Label and coreactant come into contact with clean electrode

- Electron-transfer to label and coreactant occurs through a thick organic layer

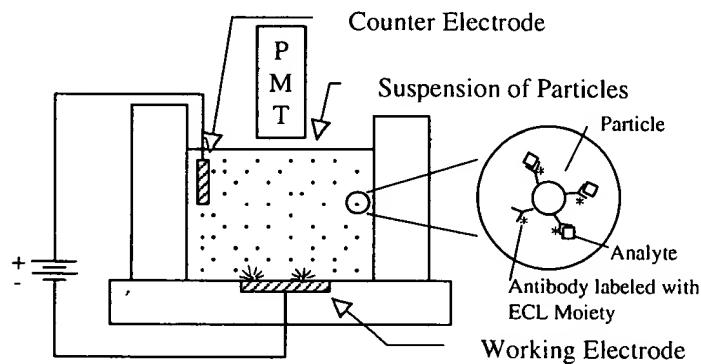
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Leventis:



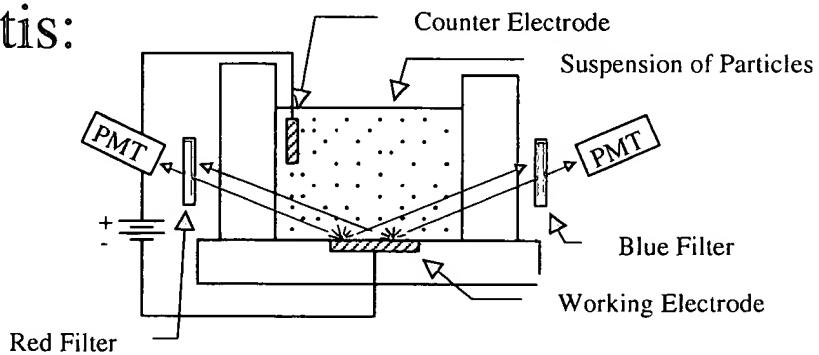
- Distinguishes between ECL moieties on the basis of color.
- ECL moieties are in solution and diffuse to the electrode.

Shibue:



- Binding of analyte decreases ECL signal from ECL moieties on antibody immobilized on particle.
- ECL moieties are in suspension and diffuse to the electrode.

Shibue + Leventis:

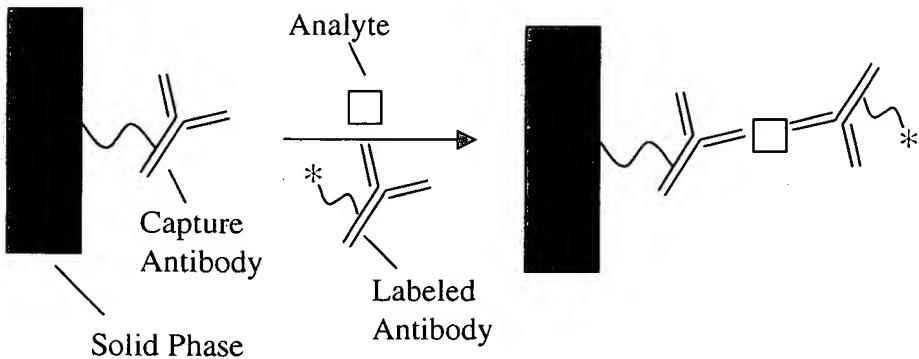


- Binding of analyte decreases ECL signal from ECL moieties on antibody immobilized on particle.
- ECL moieties are in suspension and diffuse to the electrode.
- Analytes distinguished on the basis of color.



FIG. 6

Solid Phase Binding Assays



*Binding events measured through
the use of a detectable label (*).*

<i>Detection Technique</i>	<i>Label</i>
Radioactivity Measurement	Radioisotopes: ^{125}I , etc.
Fluorescence Measurement	Fluorophore: Fluorescein, etc.
Agglutination	Particle: Latex Bead, etc.
ELISA	Enzyme: Alkaline Phosphatase, etc.
Electrochemiluminescence	ECL Moiety: $\text{Ru}(\text{bpy})_3^{2+}$, etc.